REMARKS

The inventors have made a significant contribution to the field of cancer therapy by identifying a cancer target and showing that reducing expression of the gene product inhibits tumor cell growth. Accordingly, the invention is drawn to inhibiting tumor growth by contacting tumor cells with aspartyl beta hydroxylase (AAH) antisense nucleic acids.

Claims 10, 13-15, and 39-71 are pending. The amendments to claims 10, 43, 51, and 60 as well as new claims 69-71, are supported by disclosure throughout the specification, e.g., at page 3, lines 23-29; page 4, lines 4-14; and page 14, lines 13-15.

No new matter has been added by this amendment.

35 U.S.C. § 112, first paragraph

Claims 10, 13-15, and 39-68 were rejected for overbreadth and lack of enablement. In the paragraph spanning pages 2-3 of Paper No. 18, the Examiner states:

> Demonstrating the inhibition of aspartyl beta hydroxylase expression in tumor cells cannot alone support the predictability of the method for prevention of or treating said tumor growth through administration of either an antisense nucleic acid or an intrabody directed to aspartyl beta hydroxylase. Tumor growth is a complex and multiple step process that proceeds by the acquisition of successive genetic insults (A. Hagemeijer, Leukemia, 1992, Vol. 6, Suppl. 4, pp. 16-18). The establishment and growth of a tumor is subject to variables beyond the overexpression of a single enzyme. The ability of a host to suppress and thereby prevent the tumor from establishing itself will vary depending upon factors such as the condition of the host, the type and stage of tumor and tumor burden.

The pending claims are drawn to methods of inhibiting tumor cell growth. The claims do not recite preventing or treating. As was discussed in the previous amendment, the claims no

longer encompass an intrabody. Applicants have provided evidence demonstrating that contacting several different types of tumor cells with an AAH antisense construct results in inhibition of growth of the tumor cells. The pending claims are commensurate with the scope of the disclosure and require a 5' regulatory region or coding region in exon 1 of an AAH gene.

Applicants do not dispute that "tumor growth is a complex and multiple step process that proceeds by the acquisition of successive genetic insults". However, identification of each individual "step" or "insult", i.e., new a cancer target, and how to manipulate the cancer target represents an invention and a significant contribution to the field of cancer therapy. In this case, the cancer target (AAH) was defined and manipulating its expression (i.e., inhibiting AAH expression) was shown to inhibit tumor cell growth. Although many factors may contribute to "establishment and growth of a tumor", the present invention targets at least one of those factors and leads to a benefit of inhibiting tumor growth. It does not preclude other approaches of treating cancer, and indeed may be combined with other methods which target other "steps" or "insults". Applicants have demonstrated inhibition of AAH by antisense oligonucleotides inhibits tumor cell growth and migration (See Delcaration of Dr. Jack Wands, July 19, 2001). These data indicate that the claimed methods of contacting tumor cells with AAH antisense nucleic acids inhibit tumor growth, and therefore, represent one reliable and predictable method of reducing tumor cell growth.

With respect to administration of an antisense nucleic acid *in vivo*, the Examiner states:

> It is recognized in the art that the development of clinically useful antisense strategies for disease therapy is fraught with difficulties, even when the nucleic acid sequence for the target protein is known.

The Examiner's rationale seems to suggest that methods for using antisense oligonucleotides to inhibit gene expression (or other activities), even if limited to a specific gene target, are not worthy of patent protection because of difficulties in the field. Although antisense methods may require further experimentation to optimize the desired result of reduced expression of a target gene, the effectiveness of the methodology is well established and well accepted by the scientific and medical community. Numerous antisense antisense compositions are currently being administered to human subjects and antisense technology is regarded as a sound therapeutic approach.

The Examiner discusses various caveats to the general approach of antisense technology to inhibit expression of a target gene. For example, the Examiner cited to A. Branch (Hepatology, 1996, Vol. 24, pp. 1517-1529) stating "antisense nucleic acids, such as antisense cDNA or antisense exons, that are large and highly charged ofter interact with a wide variety of untargeted cellular components causing undesirable 'non-antisense' effects". The claims have been amended to distinguish the invention from Branch's "conventional antisense nucleic acids [which] are highly charged, complex molecules that interact with a wide variety of unintended cellular and microbial components". The antisense molecules now required by the claims are small (10-50 nucleotides in length) nucleic acids with a nucleotide sequence that corresponds to a 5' regulatory or coding region of exon 1 of an AAH gene. Therefore, the nucleic acids of the claimed methods are not subject to the problems described by Branch. Moreover, Branch provides guidelines for designing and evaluating antisense molecules that inhibit gene expression as desired, thereby providing a practioner with guidance regarding implementing antisense technology for the purpose of inhibiting gene expression.

Citing to Broaddus et al., (Methods in Enzymology, 2000, Vol. 314, pp. 121-135), the Examiner states:

> Broaddus teaches that a highly empirical approach to testing of candidate anti-sense oligonucleotides is critical for the establishment of an antisense oligonucleotide as a therapeutic agent for the treatment of patients. This requirement has not been met by the instant specification, therefore, one of skill in the art would be forced into undue experimentation without reasonable expectation of success in order to practic the invention as claimed.

The enablement standard permits some experimentation, so long as it is not undue. The difficulties described by the Examiner pertain to the general technology of antisense, rather than the specific invention claimed – use of AAH antisense nucleic acids to inhibit tumor growth. Once a target gene has been identified, identification of optimal oligonucleotides is standard experimentation in the field of antisense technology. In the present case, not only have the Applicants identified the target gene, AAH, they have identified specific regions, e.g,. 5' regulatory sequences, of the target gene to which the inhibitory antisense oligonucleotides bind.

Applicants have undertaken an empirical approach to defining antisense oligonucleotides that work to inhibit gene expression, and in turn, tumor cell growth. The amended claims reflect this analysis and require specific discrete regions of an AAH gene (exon 1) to which the antisense sequences correspond. Further experimentation is therefore limited to sequences in exon 1 of an AAH gene. The data presented in Dr. Wands' Declaration demonstrate that 4 different exon 1 AAH antisense nucleic acids within the claims predictably and reliably inhibit AAH gene expression as well as tumor cell growth and migration. Optimization of exon 1 oligonucleotides and confirming their inhibitory activity *in vivo* is well within the skill of a practitioner in the art of antisense technology. Given the description in the specification, evidence that exon 1 AAH oligonucleotides inhibit tumor cell growth, and the copious

information regarding making and using antisense oligonucleotides known in the art, Applicants submit that undue experimentation is not required to practice the invention as now claimed.

CONCLUSION

On the basis of the foregoing amendments and remarks, Applicants respectfully submit that the pending claims are in condition for allowance.

Applicants file concurrently herewith a petition for a two (3) month extension of time, together with a check for $930.00 to cover the fee pursuant to 37 C.F.R. § 1.17(a)(3). With the extension, this amendment is due on or before February 13, 2003. The Commissioner is hereby authorized to charge same, or credit any overpayment, to Deposit Account No. 50-0311 (Reference No. 21486-032).

Respectfully submitted,

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EXHIBIT A

Marked up Version

In the claims:

10. A method of inhibiting tumor growth in a mammal comprising administering to said mammal a compound which inhibits expression of alpha-ketoglutarate-dependent dioxygenase aspartyl (asparaginyl) beta-hydroxylase (AAH), wherein said compound is a AAH antisense nucleic acid comprising a sequence which is complementary to a 5' AAH regulatory sequence, said nucleic acid comprising 10-50 nucleotides in length.

13. The method of claim 10, wherein said tumor is derived from endodermal tissue.

14. The method of claim 10, wherein said tumor is selected from the group consisting of colon cancer, breast cancer, pancreatic cancer, liver cancer, and cancer of the bile ducts.

15. The method of claim 10, wherein said tumor is a CNS tumor.

39. The method of claim 10, wherein said tumor is a glioblastoma.

40. The method of claim 10, wherein said tumor is a neuroblastoma.

41. The method of claim 10, wherein said tumor is a cholangiocarcinoma.

42. The method of claim 10, wherein said tumor is a hepatocellular carcinoma.

43. A method of inhibiting tumor growth in a mammal comprising administering to said mammal a HAAH antisense nucleic acid, wherein said nucleic acid comprises a sequence which is complementary to a 5' portion of an AAH coding sequence, said nucleic acid comprising 10-50 nucleotides in length.

44. The method of claim 43, wherein said tumor is derived from endodermal tissue.

45. The method of claim 43, wherein said tumor is selected from the group consisting of colon cancer, breast cancer, pancreatic cancer, liver cancer, and cancer of the bile duct.

46. The method of claim 43, wherein said tumor is a CNS tumor.

47. The method of claim 43, wherein said tumor is a glioblastoma.

48. The method of claim 43, wherein said tumor is a neuroblastoma.

49. The method of claim 43, wherein said tumor is a cholangiocarcinoma.

50. The method of claim 43, wherein said tumor is a hepatocellular carcinoma.

51. A method of inhibiting tumor growth in a mammal comprising administering to said mammal a AAH antisense nucleic acid, wherein said nucleic acid comprises a sequence which is

complementary to a AAH sequence encoding a signal peptide, said nucleic acid comprising 10-50 nucleotides in length.

52. The method of claim 51, wherein said tumor is derived from endodermal tissue.

53. The method of claim 51, wherein said tumor is selected from the group consisting of colon cancer, breast cancer, pancreatic cancer, liver cancer, and cancer of the bile duct.

54. The method of claim 51, wherein said tumor is a CNS tumor.

55. The method of claim 51, wherein said tumor is a glioblastoma.

56. The method of claim 51, wherein said tumor is a neuroblastoma.

57. The method of claim 51, wherein said tumor is a cholangiocarcinoma.

58. The method of claim 51, wherein said tumor is a hepatocellular carcinoma.

59. A method of inhibiting tumor growth in a mammal comprising administering to said mammal a AAH antisense nucleic acid, wherein said nucleic acid comprises a sequence which is complementary to a AAH sequence in exon 1 of a AAH gene, said nucleic acid comprising 10-50 nucleotides in length.

60. The method of claim 59, wherein said tumor is derived from endodermal tissue.

61. The method of claim 59, wherein said tumor is selected from the group consisting of colon cancer, breast cancer, pancreatic cancer, liver cancer, and cancer of the bile duct.

62. The method of claim 59, wherein said tumor is a CNS tumor.

63. The method of claim 59, wherein said tumor is a glioblastoma.

64. The method of claim 59, wherein said tumor is a neuroblastoma.

65. The method of claim 59, wherein said tumor is a cholangiocarcinoma.

66. The method of claim 59, wherein said tumor is a hepatocellular carcinoma.

67. The method of claim 59, wherein said nucleic acid comprises a sequence which is complementary to a full length naturally-occurring AAH transcript.

68. The method of claim 10, 43, 51, or 59, wherein said nucleic acid is a human AAH antisense nucleic acid.

Add new claims 69-71.

69. (new) A method of inhibiting growth of a tumor cell comprising contacting said tumor cell with a compound which inhibits expression of alpha-ketoglutarate-dependent dioxygenase aspartyl (asparaginyl) beta-hydroxylase (AAH), wherein said compound is a AAH

antisense nucleic acid comprising 10-50 nucleotides and a sequence which is complementary to a AAH sequence in exon 1 of a AAH gene and wherein said tumor cell overexpresses AAH compared to a normal noncancerous cell.

70. The method of claim 69, wherein said tumor is selected from the group consisting of colon cancer, breast cancer, pancreatic cancer, liver cancer, and cancer of the bile ducts.

70. The method of claim 69, wherein said tumor is a CNS tumor.